

REMARKS/ARGUMENTS

I. Amendments to the Specification:

The Examiner contends that the sequence CAGGTAAAGT identified as a fragment of SEQ ID NO:13 set forth in the last amendment to page 31, line 29 through page 32, line 12 does not appear in SEQ ID NO:13. Applicant appreciates the Examiner's identification of an inadvertent error that has been corrected in the present amendment.

The present specification discloses a synthetic intron comprising a 5' splice site, a branch point, and a 3' splice site. *See* page 31, line 29 through page 32, line 12. The 5' splice site corresponds to residues 1-9 of SEQ ID NO:10 or residues 1-9 of SEQ ID NO:13; the branch point corresponds to residues 16-22 of SEQ ID NO:10 or residues 93-99 of SEQ ID NO:13; and the 3' splice site corresponds to residues 25-45 of SEQ ID NO:10 or residues 102-122 of SEQ ID NO:13. *Id.* SEQ ID NO:10 as originally submitted erroneously failed to show the clearly disclosed track of 77 random nucleotides located between the 5' splice site and the branch point, whereas SEQ ID NO:13 depicts the same intron including a track of 77 random nucleotides. *See* page 34, lines 17-19. SEQ ID NO:10 and SEQ ID NO:13 are fully disclosed and supported by the originally filed specification. Paragraph 90 has been amended to describe an exemplary synthetic intron in terms of SEQ ID NO:13. Accordingly, Applicant submits that no new matter has been added to the amended paragraph 90.

The Examiner also contends that SEQ ID NO:18 and 19 constitute new matter that do not have explicit or implicit support in the originally filed specification. Applicant respectfully traverses.

Applicant submits that SEQ ID NO:18 and 19 are explicitly and/or implicitly supported in the originally filed specification. The specification teaches a consensus 3' splice site sequence Y₁₁NYAGG which can be modified by extending the polypyrimidine tract Y₁₁ to 16 bases. Accordingly, the new sequence becomes Y₁₆NYAGG (SEQ ID NO:18). *See* page 33, lines 13-19. The specification also teaches that the 3' splice site consensus sequence can be modified by having the 16 base polypyrimidine tract include 7 consecutive T residues as shown in intron OPTIVS8. *See* page 33, lines 13-20. An example of the latter modification will be

TTCTTTTTTCTCTTCNYAGG (SEQ ID NO:19). Hence, Applicant submits that SEQ ID NO:18 and 19 are explicitly and/or implicitly supported in the originally filed specification.

II. Amendments to the claims

Claim Objection

Claim 51 is objected to for containing typographical error. Claim 51 has been amended to correct the error.

Rejection Under 35 U.S.C. §112

Claims 5, 10, 14, 50-55, 65-71 and 73-76 are rejected under 35 U.S.C. §112, first paragraph, as containing new matter. This rejection is respectfully traversed.

Claims 5, 10, and 14 have been amended to recite a plasmid containing one or more synthetic intron comprising a 5' splice site having the sequence of residues 1-9 of SEQ ID NO:13, a branch point having the sequence of residues 93-99 of SEQ ID NO:13, and a 3' splice site having the sequence of residues 102-122 of SEQ ID NO:13. Applicant submits that the claims as amended are fully supported by the originally filed specification, and no new matter has been added. *See* page 31, line 29 through page 32, line 12.

Claims 52-53 have been amended to recite a plasmid wherein the 3' splice site is weakened with respect to the alternative 3' splice site. Support for this limitation can be found on page 38, line 21 to page 39, line 6.

Claim 65 has been amended to recite an intron comprising a 5' splice site having the sequence of SEQ ID NO:15, a branch point having the sequence of SEQ ID NO:17, and a 3' splice site having the sequence of SEQ ID NO:18, wherein the 3' splice site contains 7 consecutive T residues. As discussed above, Applicant submits that the instant specification teaches a consensus 3' splice site sequence Y₁₁NYAGG which can be modified by extending the polypyrimidine tract Y₁₁ to 16 bases. Accordingly, the new sequence becomes Y₁₆NYAGG (SEQ ID NO:18). *See* page 33, lines 13-20. The specification also teaches that the 3' splice site consensus sequence can be modified by having the 16 base polypyrimidine tract include 7 consecutive T residues as shown in intron OPTIVS8. *See* page 33, lines 13-20. Accordingly,

Applicant submits that the claim as amended is fully supported by the originally filed specification, and no new matter has been added.

Claim 67 is rejected for reciting new matter TTCTTTTTTCTCTTCNYAGG (SEQ ID NO:19). Applicant respectfully traverses. As discussed above, the specification teaches a consensus 3' splice site sequence Y₁₁NYAGG which can be modified by extending the polypyrimidine tract Y₁₁ to 16 bases. Accordingly, the new sequence becomes Y₁₆NYAGG (SEQ ID NO:18). *See* page 33, lines 13-19. The present specification further teaches that the 3' splice site consensus sequence can be modified by having the 16 bases polypyrimidine tract include 7 consecutive T residues as shown in intron OPTVS8. *See* page 33, lines 13-20. An example of the latter modification will thus be TTCTTTTTTCTCTTCNYAGG (SEQ ID NO:19).

Claims 70 and 75 are rejected for reciting new matter "a branch point located 24 to 38 nucleotides upstream from a site of splicing in the 3' splice site". Applicant respectfully traverses. As acknowledged by the Examiner, the specification teaches a branch point is typically located 18-38 nucleotides upstream of a 3' splice site, and the branch point in the exemplary synthetic intron OPTVS8 is 24 nucleotides upstream from the 3' splice site. Hence, one of ordinary skill in the art would readily recognize that the specification is explicitly and/or implicitly teaching a branch point located in the range of 18-38 nucleotides upstream of a 3' splice site, and the range recited in claims 70 and 75 is within the range disclosed in the specification.

Claim 73 is rejected for reciting new matter SEQ ID NO:18. Claim 73 has been amended to recite a 3' splice site having the sequence of SEQ ID NO:18, wherein the 3' splice site contains 7 consecutive T residues. As discussed above, Applicant submits that SEQ ID NO:18 containing 7 consecutive T residues is explicitly and/or implicitly supported in the originally filed specification.

Claims 73-75 are rejected for reciting a synthetic intron from about 90 to 200 nucleotides in length. As acknowledged by the Examiner, the specification teaches most naturally occurring introns are 90-200 nucleotides in length, and the length of the disclosed exemplary synthetic intron OPTVS8 also falls within this range. Hence, Applicant submits that one of ordinary skill

in the art would readily recognize that the specification is explicitly and/or implicitly teaching a synthetic intron with a length of 90-200 nucleotides.

Claims 50 and 55 have been canceled. In view of the above remarks, Applicant respectfully requests that the rejection of claims 5, 10, 14, 51-54, 65-71 and 73-76 under 35 U.S.C. §112, first paragraph, be withdrawn.

Rejection of Claim 5 Under 35 U.S.C. §103

Claim 5 is rejected under 35 U.S.C. §103(a) as being unpatentable over either Mascarenhas et al. (Plant Molecular Biology 15:913 (1990)) or Petitclerc et al. (J. Biotechnology 40:169 (1995)) in view of any one of Mulvihill et al. (U.S. Patent 5,648,254), Carrano et al. (U.S. Patent 5,739,118) or Ligon et al. (U.S. Patent 5,723,759). To the extent that these references are further considered to be applicable to the amended claims, the rejection is respectfully traversed.

All of the claims are drawn to use of synthetic introns of defined sequence that differ from those arguably disclosed in the cited references.

Mascarenhas et al. teach insertion of native maize alcohol dehydrogenase-1 introns 2 or 6 into a 5' untranslated region for enhanced expression of a reporter gene. Although the Mascarenhas reference does not disclose the sequence of these introns, as shown below, a review of the sequences reveals that they differ from the claimed introns. This analysis is based on the GenBank record for maize adh1(ZMADH1SA VERSION X04049.1 GI:22123, Copy provided in accompanying IDS, with IVS2 and IVS6 highlighted). Note that although the location of the 5' and 3' splice sites are clear, the location of the branch point is less certain, and is therefore approximated)

ZMADH1SA Intron number 2 identified at 1907 – 2003:

aag[^]gtatctaatcagccatcccatttgtgatctttgtcagtagatatgatacaacaactcgc**ggttgact**tgccgctt
cttggcg**gcttatctgtctcag[^]g**

ZMADH1SA Intron number 6 identified at 3135 – 3476:

aag[^]gtacagtacacacacatgtatatatgtatgatgtatcccttcgatcgaaggcatgccttggtataatcactgagtagtcatt
ttattactttgttttgacaagtcagtagttcatccatttgtcccatTTTTTcagcttggaagtttggttgactggcacttggtct
aataactgagtagtcattttattacgttgtttcgacaagtcagtagctcatccatctgtcccatTTTTTcagctaggaagtttggt
tgcactggccttggaactaataactgattagtcattttattacattgttttcgaca**aagtcagtagctcatccatctgtcccatTTTTTc**
ag[^]c

In the comparison below, Mascarenhas Adh1 IVS2 in small type, is sandwiched with the claimed sequences in all caps and reveals a lack of identity with the claimed sequences:

CAG [^] GTAAGT (SEQ.ID.13/1-9)	TACTAAC (SEQ.ID.13/93-99)	
aag [^] gtatct	-N ₅₃ -	ggttgacttgcgcccttcttggcggttatctgtctcag[^]g (Adh1 IVS2)
		YYYYYYYYYYYYYYYYNYAG [^] G (3' splice
		Y ₁₆ NYAG [^] G, Y=C/T,N=any, including 7
		consecutive T residues, SEQ.ID.18)

In the comparison below, Mascarenhas Adh1 IVS6 in small type, is sandwiched with the claimed sequences in all caps and reveals a lack of identity with the claimed sequences:

CAG [^] GTAAGT (SEQ.ID.13/1-9)	TACTAAC (SEQ.ID.13/93-99)	
aag [^] gtacag	-N ₃₀₁ -	aagtcagtagctcatccatctgtcccatcttttcag[^]c (Adh1 IVS6)
		YYYYYYYYYYYYYYYYNYAG [^] G (3' splice
		Y ₁₆ NYAG [^] G, Y=C/T,N=any, including 7
		consecutive T residues, SEQ.ID.18)

Petitclerc et al. teaches use of an intron from SV40 late genes or a synthetic intron generated by the association of an adenovirus splice donor and an immunoglobulin G splice acceptor to express foreign DNA efficiently in different cell lines. Although not given in the Petitclerc article, the SV40 VP1 intron sequence was known. Referring to the GenBank reference sequence NC_001669.1 GI:9628421, copy provided in the accompanying IDS) the VP1 intron is located at region 527 – 1462 and is depicted below with underlining of the 5' splice donor, branch and 3' splice acceptor.

ctg[^]gtaagtttagtctttttgtcttttatttcagggtccatggtgctgctttaaactgttgggggacctaattgctactgtgtc
tgaagctgctgctgctactggattttcagtagctgaaattgctgctggagaggccgctgctgcaattgaagtgcaactgcatctg
ttgctactgttgaaggcctaacaacctctgaggaattgctgctataggcctcactccacaggcctatgctgtgatctctgggct
cctgctgctatagctggatttgcagctttactgcaaactgtgactggtgtgagcgctgttgctcaagtggggtatagatttttag
tgactgggatcacaagtttctactgttgggttatatcaacaaccaggaatggctgtagatttgtataggccagatgattactatg
atatatttatttcctggagtacaaaccttgttcacagtgttcagtatcttgacccagacattgggggtccaaactttttaatgcc
atttctcaagctttttggcgtgtaatacaaaatgacattccttaggctcacctcacaggagcttgaaagaagaacccaaagatattt
aaggagcagtttggcaagggttttagaggaaactacttggacagtaattaatgctcctgttaattggtataactctttacaagatt
actactctactttgtctcccattaggcctacaatggtgagacaagtagccaacagggaagggttgcaaatatcatttgggcacacc
tatgataatattgatgaagcagacagtagttcagcaagtaactgagaggtgggaagctcaaagccaaagtcctaattgtgcagtcagg
tgaatttattgaaaaatttagggtcctggtggtgcaaatcaaagaactgct**cctcagtg**gatgtt**gcctttacttctag[^]g**

A comparison of SV40 VP1 in small type below and sandwiched with the claimed sequences in all caps reveals a lack of identity with the claimed sequences:

```
CAG^GTAAGT (SEQ.ID.13/1-9)      TACTAAC (SEQ.ID.13/93-99)
ctg^gtaagt      - N901-      tcctcagtgatgttgccctttacttctag^g (SV40 VP1 intron)
                                   YYYYYYYYYYYYYYNYAG^G (3' splice
                                   Y16NYAG^G, Y=C/T,N=any, including 7
                                   consecutive T residues, SEQ.ID.18)
```

The Petitelerc reference does not provide a sequence for the described synthetic intron, which was generated by the association of an adenovirus splice donor and an immunoglobulin G splice acceptor. Although a full sequence does not appear to be readily available, given that the present claims relate to a specific novel 3' splice acceptor, the obviousness issue is resolvable by reference to the sequence of the 3' immunoglobulin G splice acceptor (referenced in the article as Kaufman, Molecular and Cellular Biology 2 (1982) 1304, included herewith in the accompanying IDS, which in turn references Bothwell, A.L., et al Cell 24 (3), 625-637 (1981) (copy not included but sequence provided in GenBank ACCESSION J00537 GI:196161, of which a copy is provided in the accompanying IDS). The immunoglobulin intron is identified as VH-102 intron A, REGION: 47..128).

```
LOCUS  MUSIGHWH                      527 bp    DNA        linear    ROD 27-APR-1993
DEFINITION  Mouse Ig germline H-chain, C57Bl/6 b-NP-related gene VH102, VH-II,  5'
end.
VERSION     J00537.1  GI:196161
intron      47..128
              /note="VH-102 intron A"
```

```
atgggatggagctgtatcatcctcttcttgtcagcaacagctacaggtgaaggggctcacagtagcaggcttgaggtctggccatat
acatgggtgacaatgacatccacttttgccctttctctccacaggtgtccactcccatgtccaactgcagcagcctggggctgagctg
gtgaagcctggggcttcagtgaaggtgtcctgcaaggcttcttggtacaccttcaccagctactggatgcactgggtgaagcagag
gcttgccaaggccttgagtggaattggaaggattcatccttctgatagtataactacaatcaaaagttcaagggcaaggcca
cattgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaggactctgcggtctattactgtgca
atacacagtgttgtaaccacatcctgagagtgtcagaaacctggatgagtagcaaaactgccttgagactgaggagactcagagaa
ggtttgcttgt
```

A comparison of the Ig intron in small type below and sandwiched with the claimed sequences in all caps reveals a lack of identity with the claimed sequences:

```
CAG^GTAAGT (SEQ.ID.13/1-9) TACTAAC (SEQ.ID.13/93-99)
cag^gtaagg      - N44-      caatgacatccacttttgccctttctctccacag^g "Ig VH-102 intron A"
                                   YYYYYYYYYYYYYYNYAG^G (3' splice Y16NYAG^G,
                                   Y=C/T,N=any, including 7 consecutive T
                                   residues, SEQ.ID.18)
```

Mulvihill et al. teach a method of introducing a first DNA encoding a protein of interest into a eukaryotic cell, followed by introducing an additional DNA encoding a protein that would

process or stabilize the protein of interest. Mulvihill does not disclose any sequences but does suggest the incorporation of RNA splice signals into the vector construct, preferably obtained from adenovirus and/or immunoglobulin genes (Col. 8, lines 34 – 38). As discussed above in reference to the Petitclerc reference, Mulvihill's use of adenovirus and/or immunoglobulin genes does not teach or suggest use of the claimed synthetic intron, which in particular contains a 3' acceptor sequence which is modified from consensus by inclusion of 7 contiguous T residues..

Carrano et al. teach a nucleic acid molecule encoding one or more epitopes of a pathogen antigen. Carrano mentions that some embodiments that include rev have a splice acceptor upstream of the start codon for rev, while constructs that contain gag have a splice donor upstream of the gag translational start codon. (See Col. 22, lines 25 – 28). In preferred embodiments, expression plasmids include HIV-1 genes and splice junctions in their native form. See Col. 24, lines 23 – 25. In other embodiments, the SV40 polyA site and early splicing region obtained by PCR of pCEP4 are included. Col. 34., line 62. Alternative splicing is suggested at the end of Example 35 for expression of multiple proteins but without any disclosure of splice sequences. Use of a HIV splice acceptor is mentioned in Example 36 and the HIV major splice donor in Example 41. All of these comments are without disclosure of sequences. Referring to GenBank HIV-1 reference sequence, LOCUS HIVHXB2CG VERSION K03455.1 GI:1906382 (copy included in the accompanying IDS), two introns are identified, at 744 – 5777 and 6046 – 8378. The 5' major splice donor sequence is believed to be the first intron from which the following 5' donor and 3' acceptor sequences are shown below in small type together with the claimed 5' donor and 3' acceptor sequences in all caps:

```
721 caagagggcga ggggcggcga ctg^gtgagta cgccaaaaat ttgactagc . . .  
CAG^GTAAGT (SEQ.ID.13/1-9)
```

```
5761 tgtttatcca ttttcag^aat tgggtgtcga catagcagaa taggcgttac tcgacagagg  
YYYYYYYYYYYYYYYYNYAG^G (3' splice Y16NYAG^G, Y=C/T,N=any, including 7  
consecutive T residues, SEQ.ID.18)
```

The 5' donor and 3' acceptor sequences of the second intron are shown below in small type together with the claimed 5' donor and 3' acceptor sequences in all caps:

```
6001 agtcatcag aacagtcaga ctcatcaagc ttctctatca aagca^gtaag tagtacatgt  
CAG^GTAAG T (SEQ.ID.13/1-9)
```

```
8341 atagagttag gcagggatattcaccattatcgtttcag^ac ccacctccca accccgaggg  
YYYYYYYYYYYYYYYYNYAG^G (3' splice Y16NYAG^G,  
Y=C/T,N=any, including 7 consecutive T residues, SEQ.ID.18)
```

As is clear from these comparisons, the claimed sequences are not taught or suggested by the cited Carrano reference.

Ligon et al. disclose genes required for recombinant production of antipathogenic substances in a plants. The specification discloses use of native plant intron sequences, including from the above discussed Adh1 maize gene. No sequences are identified for these introns.

The Examiner should note that the prior art references when combined must teach or suggest all the claim limitations of the claim to establish a *prima facie* case of obviousness. MPEP §2142. Accordingly, absent any teaching or suggestion on a synthetic intron comprising the sequences disclosed and claimed herein, the cited references do not render claim 5 obvious.

Applicant reiterates that a *prima facie* case of obviousness must be established by showing the cited references when combined teach or suggest all the claim limitations of the instant claim. The Examiner has not provided any evidence or proof from any prior art reference that teaches the sequences of the instant claim. Furthermore, as presently amended, all claims require the 3' splice site Y₁₆NYAG[^]G, Y = C/T, N = any nucleotide, including 7 consecutive T residues, SEQ.ID.18), which is an engineered sequence that does not appear to be present or suggested in any of the cited references.

In view of the above remarks, Applicant respectfully requests that the rejection of claim 5 under 35 U.S.C. §103(a) be withdrawn.

Rejection of Claim 10 Under 35 U.S.C. §103

Claim 10 is rejected under 35 U.S.C. §103(a) as being unpatentable over Mascarenhas et al. (Plant Molecular Biology 15:913 (1990)) or Petitclerc et al. (J. Biotechnology 40:169 (1995)) in view of Mulvihill et al. (U.S. Patent 5,648,254), Carrano et al. (U.S. Patent 5,739,118) or Ligon et al. (U.S. Patent 5,723,759), and further in view of Zitvogel et al. (Human Gene Therapy 5:1493 (1994)). The rejection is respectfully traversed.

Claim 10 is drawn to a plasmid carrying a synthetic intron comprising a 5' splice site having the sequence of residues 1-9 of SEQ ID NO:13, a branch point having the sequence of residues 93-99 of SEQ ID NO:13, and a 3' splice site having the sequence of residues 102-122 of SEQ ID NO:13.

Mascarenhas et al., Petitcherc et al., Mulvihill et al., Carrano et al., and Ligon et al. have been discussed above. Zitvogel et al. teach the construction of retroviral vector expressing biologically active human interleukin-12 and does not appear to recite use of specific introns, if any.

The Examiner essentially rejects claim 10 with the same argument in the rejection to claim 5. None of the cited references teach or suggest a synthetic intron comprising a 5' splice site (residues 1-9 of SEQ ID NO:13), a branch point (residues 93-99 of SEQ ID NO:13), and a 3' splice site (residues 102-122 of SEQ ID NO:13) as claimed herein. Consequently, Applicant respectfully requests that the rejection of claim 10 under 35 U.S.C. §103(a) be withdrawn.

Rejection of Claim 14 Under 35 U.S.C. §103

Claim 14 is rejected under 35 U.S.C. §103(a) as being unpatentable over Dirks et al. (Gene 128:247 (1993)) in view of Rautmann and Breathnach (Nature 315:430 (1985)). The rejection is respectfully traversed. Claim 14 as amended is drawn to a plasmid carrying a synthetic intron that comprises a 5' splice site (residues 1-9 of SEQ ID NO:13), a branch point (residues 93-99 of SEQ ID NO:13), and a 3' splice site (residues 102-122 of SEQ ID NO:13).

Dirks et al. teach dicistronic vectors utilizing the internal ribosomal entry site sequences of poliovirus including with use of an SV40 splice donor/acceptor. One of the disclosed plasmids pSBC1 has a corresponding GenBank record (LOCUS PSBC1 DEFINITION Bicistronic transcription unit (pSBC-1)VERSION X68257.1 GI:58272, a copy of which included in the accompanying IDS). The SV40 Splice Donor (SD)/ Acceptor (SA) are identified in the cited reference between XhoI at 356 and Eco R1 at 425. From this the SD/SA have been located and compared with the claimed sequence.

```
301 ttccagaagt agtgaggagg ctttttttga ggcctaggct ttgcaaaaa gctccctcga
361 ggaactggaa aaccagaaag ttaactg^gta agtttagtct ttttgccttt tatttcag^gt
421 cccggaattc gagctcgccc ggggatcctc tagagtcgac ctgcagaagc ttttaaaaca
```

A comparison of the SV40 VP2 intron in small type below and sandwiched with the claimed sequences in all caps reveals a lack of identity with the claimed sequences:

```

                                CAG^GTAAGT (SEQ.ID.13/1-9)
361      ggaactggaaaaccagaaagttaactg^gtaagtttagtccttttgccttttatttcag^gt
                                YYYYYYYYYYYYYYYYNYAG^G
                                (3' splice Y16NYAG^G, Y=C/T,N=any, SEQ.ID.18)
```

Rautmann and Breathnach discusses the consensus yeast branch point TACTAAC, which is the same as the instant branch point of one embodiment, as essential for splicing it its context. However, Rautman teaches the influence of different mammalian branch point sequences in the context of defined donor and acceptor sequences. The Rautmann donor and acceptor sequences are set out below in small type compared with the claimed splice donor and acceptor:

```

                                CAG^GTAAGT (SEQ.ID.13/1-9)
5' splice donor      ggatccag^gtaagctt
                                CAG^GTAAGT (SEQ.ID.13/1-9)
3' splice acceptor  gatccccttttttctag^agatct
                                YYYYYYYYYYYYYYYYNYAG^G
                                (3' splice Y16NYAG^G, Y=C/T,N=any, SEQ.ID.18)
```


The comparison above Rautmann sequences reveals a lack of identity with the claimed sequences.

Applicant submits that none of the cited references teach or suggest a synthetic intron comprising a 5' splice site (residues 1-9 of SEQ ID NO:13), a branch point (residues 93-99 of SEQ ID NO:13), and a 3' splice site (residues 102-122 of SEQ ID NO:13) as claimed herein. Absent any prior art teaching that teaches or suggests the same as disclosed herein, the claimed invention as a whole is not obvious to one of ordinary skill in the art at the time the invention was made.

Allowable Subject Matter

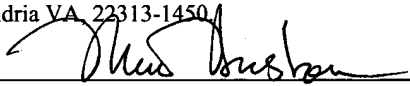
The Examiner states that claim 72 would be allowable if rewritten in independent form including all of the limitations of the base claim. Claim 72 has been rewritten in independent form including all of the limitations of the base claim.

Respectfully submitted,

By 
Hon-Man Lee

Application No. 09/754,014
Response to Final Office Action of 4/7/05

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Marilyn M. Huston	